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Functional Multipotency of Stem Cells: A Conceptual Review of Neurotrophic Factor-Based Evidence and Its Role in Translational Research

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Abstract: We here propose an updated concept of stem cells (SCs), with an emphasis on neural stem cells (NSCs). The conventional view, which has touched principally on the essential property of lineage multipotency (e.g., the ability of NSCs to differentiate into all neural cells), should be broadened to include the emerging recognition of biofunctional multipotency of SCs to mediate systemic homeostasis, evidenced in NSCs in particular by the secretion of neurotrophic factors. Under this new conceptual context and taking the NSC as a leading example, one may begin to appreciate and seek the "logic" behind the wide range of molecular tactics the NSC appears to serve at successive developmental stages as it integrates into and prepares, modifies, and guides the surrounding CNS micro- and macro-environment towards the formation and self-maintenance of a functioning adult nervous system. We suggest that embracing this view of the "multipotency" of the SCs is pivotal for correctly, efficiently, and optimally exploiting stem cell biology for therapeutic applications, including reconstitution of a dysfunctional CNS.

Keywords: Stem cells, Neural stem cells, Multipotency, Neurotrophic factors, Neural Repair, Spinal Cord Injury.

BACKGROUND

Along with refinement of our understanding on the biology and translational potential of neural stem cells (NSCs) [1-6], there has been an increasingly appreciated paradigm shift regarding how the adult mammalian central nervous system (CNS) could be repaired for functional restoration. The long-standing dogma that the adult CNS was immutable to anatomical and functional repair was challenged by an unambiguous demonstration that cells derived from the CNS from a variety of structures and at different developmental stages, including adulthood, possess stem-like properties. NSCs are primordial and uncommitted cells that have been believed to give rise to the vast array of more specialized cells of the CNS. They are operationally defined by their abilities (1) to differentiate into cells of all neural lineages (i.e. neurons of multiple subtypes, oligodendroglia, and astroglia) in multiple regional and developmental contexts (i.e., to be multipotent); (2) to self-renew (i.e., to give rise also to new NSCs with similar potential); (3) to migrate and populate developing and/or degenerating CNS regions; and

(4) to have biofunctional multipotency to mediate systemic homeostasis through capacities such as production of trophic factors, formation of gap junctions, etc. [7,8]. Since monoclonal derivation of progeny is obligatory to the definition of NSC: that is, a single cell must possess all the aforementioned attributes, which suggests the ready availability of NSCs, many studies, including ours, in the past two decades, provided hope that the use of NSCs might circumvent some limitations of presently available graft material and gene transfer vehicles and make feasible a broader range of therapeutic strategies. This significant advance has led neurobiologists to speculate how such phenomena might be further harnessed for both therapeutic advantage and for better understanding of developmental neurobiology mechanisms.

Most studies, to date, are focused principally on exploring the biologically most apparent features of NSCs in comparison to existing gene therapy and cell transplantation strategies. For this purpose, NSCs, with their homogeneous and well-defined neural differentiation capacity, have been pursued primarily as a modality that could replace dead or degenerating neural cells in a wide variety of neurological diseases and trauma. For certain pathological models of the brain, NSCs and their progeny not only could integrate with host tissue at their site of implantation [9-11], but also could interact with distant brain tissue *via* migration in response to

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chemical cues in the host [12-15]. The responsiveness of NSCs to microenvironmental cues in the surrounding CNS tissue provides them with a unique trait distinguishing them from fetal brain/spinal cord tissue and non-neural cells (e.g., fibroblasts or mesenchymal stromal stem cells [MSCs]), as well as from most viral vectors and protein infusion devices for gene delivery. For example, haematopoietic cells could not efficiently cross the blood-brain barrier and integrate throughout the CNS as effectively as NSCs. In addition, NSCs are able to “sense” the presence of degenerating neural tissue [13,14,16-18]. Conversely, effective replacement of dying or dysfunctional neurons by NSCs in the adult CNS, an ideal hypothesis the field initially entertained, has been proven to be a much more challenging endeavor, though in a few experimental models of neurodegeneration such NSC-based cell substitution appeared to be feasible in replacing dead oligodendrocytes [9,19]. We therefore have discussed here the biological perspectives of epigenetic and genetic features of rodent (in particular, murine) and human neural stem cells regarding their capacity to produce neurotrophic factors (NTFs). Also included are views of investigators in the field on how to modulate and/or augment the homeostatic function of NSCs. Finally we summarize our current thoughts about the critical roles that NSCs may play in designs for improved regimens of NTF delivery regarding neural repair and pathology correction.

NEUROTROPHIC FACTORS AND NEURAL STEM CELLS

Because NSCs are dynamic developmental entities their therapeutic potential has been studied primarily *via* strategies devised to realize desirable neural phenotypic differentiations. Much less recognized and appreciated is the fact that these developmental cells, by innate biology, produce neurotrophic factors that influence the growth and well-being of their own and surrounding tissues *via* autocrine and paracrine mechanisms. Efforts to understand this more recently recognized perspective of stem cell biology were first triggered by the observation that undifferentiated donor NSCs could exert marked neuroprotection to the spinal cord following traumatic injury, engendering a speculation that NSCs and other stem cells may have inherent capability to synthesize trophic factors [20]. Considerable research endeavors have since taken place to seek beneficial impact of stem cells (e.g., NSCs, MSCs, and embryonic stem cells [ESCs]) that are mediated mainly through soluble trophic factors and other cytokines that enable the body to reestablish homeostasis after pathologic and traumatic insults, inflammation, and tissue infarction or degeneration [21-24]. In this article, based on data mainly derived from studies on traumatic spinal cord injury (SCI), we seek to establish conceptually the biological principles of trophic factor delivery by stem cells as a novel approach to new therapeutic strategies based on developmental mechanisms [7]. For this goal, we have expanded the conceptual scope of trophic factors to include the following categories of molecules.

By our definition, neurotrophic factors, here including classic neurotrophins, are groups of naturally occurring substances that support neural cell survival, proliferation, migration, differentiation, growth, and function [25]. They

are usually proteins or simpler peptides, and are essential regulating and signaling molecules for cell development and function. Neurotrophic factors contribute to neurogenesis, and are critical in shaping neural network structure and physiological processes such as learning, memory formation, and sensorimotor function [26]. Some neurotrophic factors, conversely, can be directly involved in pathological processes [25], *via* effects on synaptic biology, dynamics of neurotransmitter release and synaptic efficacy, whereas others act through secondary messengers and various kinases [27].

Listed below are the six biological families of neurotrophic factors that directly impact stem cell development, and are expressed by natural or bioengineered stem cells. 1. Classic neurotrophins that comprise nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4); 2. The transforming growth factor (TGF)-beta family, represented by glial cell line-derived neurotrophic factor (GDNF) and the bone morphogenic proteins (BMPs); other members are neurturin, artemin, and persephin; 3. The cytokine growth factor family, including ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), and cardiotropin-1; 4. The epidermal growth factor (EGF) family, consisting of EGF, transforming growth factor-alpha (TGF-alpha), the neuregulins, and neural and thymus-derived activator for ErbB kinases (NTAK); 5. The insulin-like growth factors (IGFs), consisting of insulin-like growth factor I (IGF-I) and IGF-II; and 6. The fibroblast growth factor (FGF) family, consisting of at least 24 different proteins including acidic FGF (FGF 1) and basic FGF (FGF 2) [28,29].

For the purpose of this review, we focus our discussion only on the family of classic neurotrophins since all its members (i.e., NGF, BDNF, NT-3, and NT4/5) have been demonstrated to play critical roles in neuronal survival, differentiation, and/or function in neurotrauma settings [30]. The neurotrophins act by binding to receptor tyrosine kinases of the Trk families. NGF binds for tropomyosin-receptor-kinase-A (TrkA), BDNF and NT-4/5 bind TrkB, and NT-3 preferentially binds TrkC. Importantly, NT-3 up-regulates TrkC expression in NSCs and induces them to differentiate into neurons [31-33]. For example, Ad-TrkC can be constructed to express TrkC effectively in NSCs, enhancing NT-3-mediated neuronal differentiation, an approach that may offer additional strategies for treating neurotrauma [33]. Overexpression of TrkC in rat neural progenitor cells improves survival and reduces glial differentiation of donor cells in the intact spinal cord [34]. Neurotrophin-mediated Trk signaling plays an essential, cell-autonomous role in regulating the proliferation and differentiation of embryonic cortical precursors and thus controls cortical development at early stages [35]. Multiple alternatively spliced isoforms have been observed for TrkA, TrkB, and TrkC, especially in non-neuronal cells [31,36-38]. Some of these isoforms lack the cytoplasmic tyrosine kinase domain, but retain selective signaling and may inhibit neurite outgrowth [37,39-41]. However, brain-derived neurotrophic factor (BDNF) has proliferative effects on NSCs through the truncated TRK-B receptor [42]. Neurotrophins also bind to p75NTR. Activation of this receptor may cause cell death rather than survival, as p75NTR^{-/-} mice show reductions in neuronal cell death after

pilocarpine-induced seizures compared to wild-type [43,44]. Interestingly, the p75 neurotrophin receptor is also involved in proliferation of undifferentiated mouse ESCs, and becomes down-regulated upon ES cell differentiation [45]. NT-3 improves the neural differentiation of skin-derived precursors (SKPs) induced by retinoic acid (RA) through a p75NTR-dependent signaling pathway [46,47]. In the following sections, we will discuss how a novel concept of using prototype or engineered NSCs to deliver therapeutic neurotrophic factors was developed, based on data collected from SCI investigations, and how this concept has subsequently been used to guide administration of neurotrophic factors to treat or investigate representative neurological disorders such as SCI, Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS).

ADMINISTRATION OF NEUROTROPHIC FACTORS FOR TREATING SCI

The pharmacological use of neurotrophic factors for SCI treatment has been tested primarily with two neurotrophins: BDNF and NT-3. Unlike peripheral nerve axons, which regenerate well, the adult mammalian spinal cord appears to have an unsuccessful capacity for axonal regrowth [48,49]. Neurotrophic factors, when administered in appropriate physical and temporal gradients, have been shown to support axonal growth after peripheral nerve injury. In contrast, antagonizing these growth factors in the extracellular matrix impedes neurite outgrowth [50,51]. Due to these findings, plus the fact that molecules have been identified that can suppress axonal sprouting and regeneration in the CNS (e.g., the chondroitin sulfate proteoglycan [CSPG] molecules) [52,53], substantial experimental efforts have been given to enhance regeneration of the supraspinal descending axons through activating neurotrophic factor-mediated mechanisms that may overcome the growth inhibitory environment in the CNS [54-60]. Additional approaches include combinatorial tactics that comprise transplantation of progenitor cells into the lesion cavity with simultaneous administration of neurotrophic factors [61], or direct augmentation of key molecules of trophic factor-triggered signal transduction, such as raising intracellular cAMP levels pharmacologically [62,63]. Many of these studies report incremental improvement in axonal growth and/or motor behavior, projecting hope for advancing towards the ultimate goal of restoring function following clinical SCI.

As examples, several groups reported that BDNF can enhance growth of supraspinal motor axons into permissive growth milieus placed at sites of SCI. Both rubrospinal axons [64,65] and reticulospinal axons [66], descending networks that regulate spinal cord motor neural activities, regenerate in response to BDNF stimulation. Reversal of atrophy of neurons in the red nucleus, even one year after SCI, can occur after BDNF stimulation [67]. However, corticospinal tract (CST) axons did not respond to BDNF post-SCI, even though BDNF prevents the death of corticospinal neuronal cell bodies when directly applied to the cerebral cortex. This outcome has been attributed to the lack of BDNF receptor TrkB on CST fibers [68].

The ability of NT-3 treatment to promote regeneration of severed CST axons was also reported [69]. This triggered

additional bioengineering initiatives to enhance the delivery efficiency of NT3. Subsequently, Grill and colleagues, adapting a gene therapy strategy, demonstrated that autologous fibroblasts genetically modified to secrete NT-3 could be transplanted into the epicenter of experimental SCI to improve neural repair [70]. More recently, Tuszynski's group at UCSD reported beneficial effects of artificially established NT-3 gradients using cell-based gene delivery, increasing axonal regeneration in experimental SCI [71]. Importantly, studies comparing the therapeutic effect on axonal growth of three different methods of replenishing neurotrophic factors (direct infusion, transplantation of unmodified cells, and transplantation of genetically engineered cells with enhanced secretory function) have reported favorable results with genetically modified cells [72]. These data support the notion that using cells to administer neurotrophic factors may offer clinically favorable pharmacodynamic properties due to the interactive capability of "these functional basic units of life" [71,72]. Therefore, donor cells such as NSCs, in particular, have become highly promising candidates for delivering NTFs due to their innate biology, which regulates molecule secretion in response to environmental changes in the nervous system [7].

This viewpoint is further strengthened by observation of a therapeutic impact of endogenous NSCs on experimental SCI under the enhanced physical activity paradigm of rehabilitation that induced functional recovery. In some cases, there was a positive correlation between degrees of functional improvement and the number of nestin-expressing NSCs present in the post-lesion spinal cord [73-76], suggesting that NSC proliferation enhanced by exercise may help augment NTF production (see below for more details). Additional support comes from the facts that severe side effects of neurotrophic factors could be caused by conventional drug delivery regimens that lack biological feedback regulations, and by barriers imposed by the peptidergic structure of these trophic factors, which impair their penetration into the brain or spinal cord parenchyma, and therefore make their pharmacotherapeutic properties difficult to evaluate [77].

A NOVEL CONCEPT OF USING PROTOTYPE OR ENGINEERED NSCS TO DELIVER NEUROTROPHIC FACTORS: INSIGHTS FROM STUDIES OF ENDOGENOUS NSCS AND ACTIVITY-DEPENDENT RECOVERY FOLLOWING SCI

Endogenous NSCs in the spinal cord and their response to trauma: Mitotic activity has been known to persist in the adult mammalian spinal cord [78]. Weiss and colleagues found that the lumbosacral spinal cord normally produces the greatest number of multipotent cells, and the cervical cord the least [79]. Under most conditions, adult NSCs have astroglial characteristics and express glia fibrillary-associated protein (GFAP) [78], but retain the ability to undergo neurogenesis [80,81], and can give rise to functional neurons and glia *in vitro* and *in vivo* [82]. It has been shown that neurotrauma significantly increases the proliferative capacity of spinal cord NSCs. Ependymal zone cells that express low levels of nestin were found to be upregulated, increasing mitotic activity and generating glia, shortly after an injury to the dorsal funiculus of the spinal cord. If implanted in a pro-neurogenesis environment [74,75,83], they are also

capable of migrating and undergoing neurogenesis [81,83, 84,85].

Ependymal and parenchymal neural progenitors were also reported to be activated in response to spinal cord transection [86,87]. When isolated *in vitro*, they generate astrocytes and oligodendrocytes but few neurons. A fraction of ependymal zone cells possessing stem cell properties or oligodendrocyte progenitor cells in the parenchyma proliferate following compression and contusion injuries [84,88], and appeared to participate in repopulation of the injured areas, suggesting their potential participation in lesion repair [89,90]. This response of NSCs to the injury occurs rather rapidly; for instance, nestin was seen in ependymal cells as early as 1 day after minimal sharp instrument injury to the lateral columns [91]. This injury, however, did not supply a sufficient stimulus for ependymal zone cells to migrate to the lesion site. A relatively larger injury to the dorsal funiculus, by contrast, led to ependymal zone NSC migration from the central canal to the lesion site [82].

Since brain NSCs have been reported to respond to external stimuli such as enhanced physical activity and exercises, by increasing their proliferation, differentiation and survival [92], an extension of that rationale is to ascertain whether physical activity and exercise could serve as an independent stimulus for endogenous NSC proliferation also in the adult spinal cord. *We earlier hypothesized that a major function of proliferating NSCs in the adult mammalian CNS is the production of trophic factors and other homeostasis-maintaining molecules that are essential for endogenous healing and plasticity processes in the spinal cord* [93]. Our published data suggest that physical activity may be beneficial partially by increasing proliferation of endogenous NSCs that produce neurotrophic factors such as GDNF and BDNF, which in turn mediate neuroplasticity and improve sensorimotor functions [74]. Conversely, the contribution of neurotrophic factors to exercise-induced functional recovery *per se* has been investigated extensively. Voluntary exercise induces an up-regulation of BDNF and NT3 mRNA and protein levels in the spinal cord [94]. Increased levels of BDNF-associated mRNA and protein levels near the injury site have also been reported following treadmill training or running [94]. Additionally, direct stimulation of hindlimb stepping was noted after intrathecal administration of BDNF [95]. Other factors related to BDNF activity, such as synapsin 1, NT-3 and cyclic AMP (cAMP) response element binding protein (CREB), are also elevated in response to post-injury exercise [96].

Although injury alone may induce an increase in the levels of these neurotrophic agents [97], these astrocyte-based increases were transient (< 2 days after SCI), and thus, unlikely to facilitate later phase neuroplasticity or functional recovery. In contrast, animals that were exercised in the post-injury period demonstrated prolonged elevation of these factors, which may offer a new therapeutic window for other interventions. There is currently no agreed-upon conclusion regarding the origin of exercise-enhanced BDNF expression despite numerous studies that implemented NSCs, astrocytes, neurons and blood-born cells as neurotrophin providers under varied situations [98]. Finally, the spinal cord's inherent

regenerative potentials can also be directly stimulated by post-injury physical activity. Exercise has been shown to double the number of proliferative cells in the CNS [74,99] and to have additional benefits, such as enhanced long-term potentiation of post-synaptic efficiency [100].

Overall, experimental outcomes suggest that synapse formation and maintenance can be activity-dependent (e.g., exercise-driven), largely operated by neurotrophins [101]. Such neurotrophic drives may not only promote birth of new neurons, but also facilitate oligodendrogenesis [102]. We therefore speculate that the amplitude of NSC-based release of neurotrophic factors could be further modified by physical activity-related rehabilitation modalities and specific pharmacological treatments augmenting NSC participation and their trophic factor production that, by mimicking developmental processes, augment reciprocal therapeutic relationships with surrounding cells and cue molecules [7].

MECHANISMS THAT ENABLE THERAPEUTIC DELIVERY OF NEUROTROPHIC FACTORS FROM PROTOTYPE OR GENETICALLY ENGINEERED NSCS FOR SPINAL CORD REPAIR

It has been well recognized that NSCs have an innate ability to detect pathologic targets for trophic actions. We and other investigators previously described a critical feature of prototype NSCs that is distinct from non-NSC carriers of neurotrophin transgenes – the ability to detect pathological loci and adopt appropriate developmental initiatives toward customized trophic support or cell replacement [103,104]. These abilities were first suggested by experimental data that was derived from a clone of murine NSCs after their implantation into an adult rat spinal cord after segmental hemisection (i.e., open wound lesion), where the NSCs contributed markedly to functional restoration [20]. Moreover, when NSCs of the same lineage were transplanted into adult mouse neocortex in which pyramidal neurons of a circumscribed region were induced experimentally to undergo apoptosis, they differentiated differently than when grafted into uninjured neocortex: they preferentially differentiated into pyramidal neurons, whereas these same NSCs yielded mostly glia in normally developed neocortex [103]. These data indicate that donor NSCs can respond to biological cues in normal and abnormal microenvironments, suggesting that NSCs spontaneously have mechanisms compensating for genetic [17,105] or acquired deficiencies [14]. Though the exact mechanisms regulating the capacity of NSCs to exert developmental functions in an adult microenvironment are still unknown, it is clear that when confronted with injury or other pathologic conditions, NSCs recapitulate certain of their developmental mechanisms [24,93,103].

It is currently believed that the signals modifying exogenous and endogenous NSC production of trophic factors affect a complex group of mitogens and chemokines, trophic and tropic agents, plus adhesion and extracellular matrix molecules (i.e., common developmental signaling molecules), as well as chemotactic and angiogenic factors, activated microglia, inflammatory cells, invading macrophages and damaged neurons and glia (i.e., general inflammation-mediating components). Optimization of therapy for tissue

protection and repair in the post-mitotic environment of the adult CNS may require overcoming an insufficient supply of NSCs and endogenous neurotrophic factors. Hence, donor NSCs become attractive to consider as therapeutic supplements. However, exogenous prototype NSCs without engineered enhancements are vulnerable to same insults from secondary injury molecules as endogenous cells [20,106]. Therefore, we and our colleagues at the Langer Lab at MIT recently devised a novel chemical engineering approach to protect donor NSCs by applying antioxidant drug-releasing polymer film to alter the implantation *milieu* against neuroinflammation mediated by reactive nitrogen species (RNS). Our technology has been fruitful not only in enhancing the survival of exogenous NSCs but also in maintaining their capacity to produce neurotrophic factors such as GDNF and BDNF [107].

APPLICATION OF NTFs OR NSCs TO TREAT NEURODEGENERATIVE DISEASES

As a consideration of translational initiatives beyond SCI, the following sections will briefly discuss the possibility of using NTFs and NSCs in treatment of neurodegenerative diseases, citing studies on Alzheimer's Disease (AD) and amyotrophic lateral sclerosis (ALS) as representative examples.

The potential use of NGF, a member of the neurotrophin family, for AD therapy has been reviewed in detail [29]. NGF emerged as a candidate therapeutic molecule for neuronal degeneration soon after its discovery [111], due to its role as a vital survival factor for sensory and sympathetic neurons during development [112]. NGF also prevents both lesion-induced and spontaneous atrophy of basal forebrain cholinergic neurons, as well as ameliorating memory deficits in aged rats; both are hallmark therapeutic targets for AD [113-115]. In AD animal models NGF treatment reduced neuronal death, and simultaneously stimulated cholinergic neurons, despite exposure to a cohort of deleterious mechanisms that cause neuronal degeneration, including excitotoxicity, aging, and amyloid overproduction [116-122]. NGF treatment improved cognition in a variety of models of rodent memory dysfunction, and its neuroprotective actions have been also confirmed in non-human primate models [123-128]. Mechanistically, NGF activates Erk/MAP kinase to influence a number of growth and function-related intracellular pathways *via* TrkA receptors, and activates pro-survival gene expression and inhibits apoptosis *via* the Akt pathways [127]. The studies to date validate the essential trophic impact of NGF on adult cholinergic neurons, and corroborate that NGF is needed (and produced) throughout life in the neocortex and hippocampus [128,129].

Therefore, if the expressions of p75 and TrkA receptors remain sufficient for NGF responsiveness, NGF delivery near degenerating cholinergic neurons appears to be an appealing clinical strategy [130-133]. However, effectively delivering NGF to the brain in humans remains a challenge, primarily because the NGF protein is too large to cross the blood brain barrier (BBB). Intra-ventricular infusion can bypass the BBB in animal models, but then, NGF spreads non-selectively in the CNS, resulting in unwanted side effects such as weight loss [134], sympathetic axon sprouting

[135] and neuropathic pain related to Schwann cell invasion [136,137], despite effectiveness in the rescue of degenerating cholinergic neurons. The adverse effects were also observed in human trials [138]. Similar effects occurred in trials of patients with Parkinson's disease when GDNF or BDNF were infused intraventricularly [139,140]. These side effects observed in clinical trials highlight the necessity to establish new approaches that can overcome certain ineffective aspects of direct NTF delivery that lacks feedback regulatory mechanisms.

ALS is a fatal neurodegenerative disease with a signature pathology of progressive motor neuron death in both the spinal cord and brain, which culminates in rapid loss of muscle function and eventual respiratory failure [141,142]. The only clinically available therapy to date is riluzole (Rilutek), which marginally extends survival by limiting excitotoxicity [143] and increasing neurotrophin release from astrocytes [144]. The majority of ALS cases are sporadic, whereas only 10% are familial (FALS) [145], among which 15-20% can be traced to point mutations in cytosolic Cu²⁺/Zn²⁺ superoxide dismutase 1 (SOD1) [146].

The rapid neuronal degeneration in ALS deems neuronal replacement and neuroprotection as valuable clinical approaches to treatment. Therefore, different types of progenitor or stem cells with capacity to produce NTFs have been tested in rodent models of ALS [147-151]; these include hNT neurons derived from the human teratocarcinoma cell line, mouse Sertoli cells [147,149], human umbilical cord blood cells [147,151], human [151], or mouse [148] bone marrow transplants, [152] and mouse or human NSCs [153,154].

Data in published studies suggests, at first glance, that the lineage or site of origin of NSCs may be responsible for some of the drastic differences in therapeutic efficacy. For instance, transplantation of human NSCs isolated from fetal forebrain (i.e., HFB2050) [14] into multiple loci of the spinal cord resulted in marked increase of life span in SOD1 (G93A) mice [154], whereas neural progenitor cells (NPCs) derived from human cortex and spinal cord produced lesser results [155]. However, further analysis suggests that the NTF profiles of donor cells might have played a key role determining the therapeutic effect in rodent ALS models [154,156]. Prototype human NSCs (HFB2050) secrete a spectrum of NTFs, including BDNF and GDNF [14], whereas genetically engineered NPCs may produce high levels of one specific NTF only, which may not be adequate to counteract ALS pathophysiology that is triggered by multiple pathogenic pathways [141]. Likewise, direct expression of GDNF delivered by lentiviral vector transfection to the lumbar spinal cord showed no beneficial effects on motor neuron survival [157], nor did robust GDNF production in the spinal cord by genetically modified NPCs prevent muscle atrophy in SOD1 rats [158]. These results underscore the need to define the range of signaling mechanisms governing the interaction between stem cells and the surrounding microenvironment to reach homeostasis by regulative mechanisms such as NTFs, other secreted molecules, and direct cell-to-cell communication (e.g., *via* gap junctions). Better understanding of these processes will ultimately enable us to design combinatorial approaches with stem cell-mediated NTF therapy to tackle complex systemic pathologies such as ALS.

To the best of our knowledge, the only controlled ALS clinical trial with stem cells reported so far was performed in Italy [159], though there had been a few uncontrolled clinical studies with other growth factors, including peripheral injection, intraventricular infusion, or intrathecal injection of CNTF and BDNF [160,161]. In the phase 1 trial the effects of autologous bone marrow derived mesenchymal stem cells (MSCs) were transplanted safely in the thoracic spinal cord of 10 ALS patients [159], and no adverse effects were seen in the 24-month post-transplantation observation period. However, no significant changes in disease progression were observed. Based on recent findings in experimental animals, glial pathology in ALS should be targeted in future trials [162]. For example, ALS model mice with conditionally deleted astrocytes expressing mutant hSOD1 showed delayed disease progression [163]. By introduction of stem cells with diversified capacities to express NTFs, such as GDNF, BDNF, IGF-I or VEGF, as well as forming direct cell-to-cell communications (e.g., *via* gap junctions) [8], motor neuron survival and function may be improved through modification of the tissue microenvironment [109,154].

FUTURE DIRECTIONS

Engineering NSCs as Transgene Carriers or Biopumps

It has been well established that NSCs constitutively produce a broad range of appropriately functioning peptidergic neurotrophic and neurite outgrowth-promoting factors [104]. To augment such capabilities further, NSCs have been engineered to express various transgenes, including NT-4/5, GDNF, BDNF, NGF, L1, sonic hedgehog, wnt-1, wnt-3a, as well as an assortment of biosynthetic and metabolic enzymes. Customized implantation of genetically engineered NSCs has been used to enhance neuronal differentiation, neurite outgrowth and connectivity within diseased tissue loci [108,109]. Also, NSCs have been manipulated to express the specific TrkC receptor of a neuron-inducing neurotrophin, NT-3 [33,77]. The engineered NSCs respond to NT-3 in an autocrine or paracrine fashion [33], which appears to trigger a significantly higher percentage of differentiating neurons in the TrkC-expressing NSCs group exposed to NT-3 than in the rAd-LacZ control cell group similarly exposed to NT-3 [33].

However, attempting to intervene in the natural expression of the various neurotrophic factors in their various proportions through genetic manipulation actually appeared to cause profile changes in trophic factor expression by the engineered cells [110]. For example, enhancing expression of NT-3 in a given clone of NSCs actually extinguished the clone's expression of GDNF, replacing the promotion of motor axon ingrowth with an enhanced ingrowth of sensory axons [110]. Caution is called for when testing a therapeutic strategy based on transgene-mediated NTF overexpression in NSCs, for unintended effects may result from such (unanticipated) "disturbance" in post-engineering biological programs of the donor stem cells. The aforementioned facts, overall, suggest the feasibility of using *bona fide* NSCs or genetically engineered NSCs to serve as biopumps for a broad selection of biofactors when one is aiming to repair damaged or dysfunctional neural tissues.

Induced Pluripotent Stem Cells (iPS Cells)

The past few years have seen the swift development in pluripotent stem cell technology led by the initiating report from the Yamanaka laboratory [165], in which a group of stemness- or mitosis-related transcription factor genes (Oct4, Sox2, Klf4 and c-Myc) were introduced to transform somatic cells dramatically into stem cell-like primitive entities with pluripotency and differentiation flexibility that are, to certain degrees, similar to ESCs. However, there are serious concerns regarding the safety of such iPSCs, especially the tumorigenic consequence, which currently prevents practical clinical use of iPS cells. Significant intrinsic variability in the derived iPSCs, abnormal expression of imprinted genes due to the random integration of transcription factors, persistent donor gene expression, and difficulty in obtaining a sufficiently rapid cell multiplication rate *in vitro* to build up a cell population sufficient for use in human patients further limit the clinical use of these cells [165-167]. Therefore, additional molecular strategies using alternative gene targets [168,169], fewer targets [170-173], or viral vector-free transfection technologies have been developed [175-178], to circumvent the safety deficits of iPS cells before their therapeutic potential can be systematically examined. However, recent reports showed that patient-specific iPSCs can be generated for investigative studies on drug screening or for experimental therapy trials [179-181]. In addition, direct trans-differentiation of adult cell types has been presented as a further therapeutic option for reversing disease progression or transforming neighboring healthy cells to take over functions lost by diseased or dying cells [182]. Despite these advances, there appear to be no reports to date on NTF production by iPS cells, a pivotal parameter of stem cell biology [7], albeit there is recent important progress in the use of synthetic modified mRNA to increase efficiency of cell reprogramming and directed differentiation, as well as analyses of the impact of epigenetic memory on the molecular and functional properties of iPS cells [183-186].

A SUMMARY NOTE

Academia and the public have, in general, accepted the concept that stem cells provide an enormous opportunity to advance the understanding of developmental biology, and especially to develop novel clinical therapies. However, we still need refined knowledge on how NSCs can be crafted to deliver the range of NTFs that would meet the micro-environmental requirements to establish homeostasis that would offset an ongoing disease process. This ability of stem cells, buoyed by their innate developmental biological properties, may teach us how to mitigate the adverse side effects resulting from direct administration of NTFs one at a time. Complex diseases such as ALS, AD and Parkinson's disease are especially likely to require complex solutions. NSCs, as described in this review, can interface and work synergistically with gene and growth factor therapy, gap junction-mediated homeostasis, anti-apoptotic and neuro-protective strategies, stimulation of neurogenesis, anti-inflammatory and anti-scarring approaches, material science and tissue engineering, and at least in the case of SCI, with physical activity and exercise. Therefore, we propose an updated concept of NTF application. The traditional approach

based on the pharmacological principles of trophic factor delivery (i.e., the dose and duration of administering a particular NTF), needs be revised to incorporate the newly appreciated importance of feedback-controlled production of trophic factors by the NSCs to mediate systemic homeostasis. Under this new conceptual context, we can begin to understand

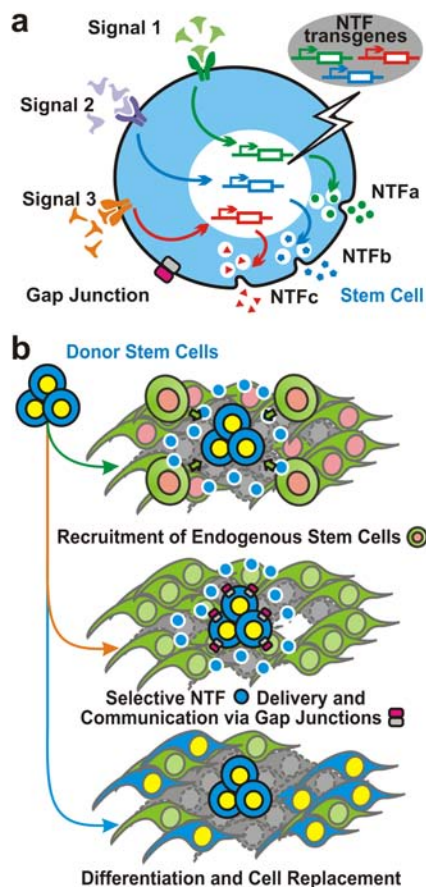


Fig. (1). Schematic summary of stem cell-based therapeutic strategies. **a**, Stem cells possess intrinsic trophic factor producing abilities, and are able to respond to environmental cues to customize the profile of trophic factor production to stage homeostasis, which, as a capacity, can be further augmented by genetically engineering the cells with extra copies of transgenes of desirable molecules. **b**, Donor stem cells, prototype or genetically modified, may provide therapeutic benefits through at least three distinct mechanisms that could cast synergistic impacts: (1) replacement of the dysfunctional or dead host cells; (2) homeostatic regulation through delivery of trophic factors in targeted manners that are biologically regulated (e.g., in response to particular environmental cues, etc.) as well as establishment of gap junctions (**b**: upper inset), etc.; and (3) recruitment of and nourishment for host endogenous stem cells. The aforementioned therapeutic mechanism No. 2, apparently, carries a wide spectrum of regulatory tactics that can be further explored to refine the trophic factor and/or other cytokine secretion at each developmental stage or neural disorder status as NSCs integrate into and prepare, modify, and guide the surrounding CNS environment towards the formation and homeostatic maintenance of a physiologically functioning adult nervous system. We believe that effectively adopting this conceptual view of the stem cell-based NTF application is essential for correct, efficient, and optimal use of trophic factors to reach their designated therapeutic goals.

the wide spectrum of molecular tactics the NSCs deploy to regulate their trophic factor secretion at each normal developmental stage as they integrate into and prepare, modify, and guide the surrounding CNS environment towards the formation and homeostatic maintenance of a physiologically functioning adult nervous system. We believe that effectively adopting this conceptual view of "functional multipotency" of stem cells is essential for correct, efficient, and optimal use of trophic factors toward therapeutic goals (Fig. 1) [7,151].

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REFERENCES

- [1] Gage, F.H. Stem cells of the central nervous system. *Curr. Opin. Neurobiol.*, **1998**, 8(5), 671-676.
- [2] Ryder, E.F.; Snyder, E.Y.; Cepko, C.L. Establishment and characterization of multipotent neural cell lines using retrovirus vector-mediated oncogene transfer. *J. Neurobiol.*, **1990**, 21(2), 356-375.
- [3] Reynolds, B.A.; Weiss, S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science*, **1992**, 255(5052), 1707-1710.
- [4] Snyder, E.Y.; Deitcher, D.L.; Walsh, C.; Arnold-Aldea, S.; Hartwig, E.A.; Cepko, C.L. Multipotent neural cell lines can engraft and participate in development of mouse cerebellum. *Cell*, **1992**, 68(1), 33-51.
- [5] Angevine, J.B., Jr.; Sidman, R.L. Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature*, **1961**, 192, 766-768.
- [6] Altman, J. Are new neurons formed in the brains of adult mammals? *Science*, **1962**, 135, 1127-1128.
- [7] Teng, Y.D.; Kabatas, S.; Wakeman, D.R.; Li, J.; Snyder, E.Y.; Sidman, R.L. Functional multipotency of neural stem cells and its therapeutic implications. In: *Perspectives of Stem Cells: From tools for studying mechanisms of neuronal differentiation towards therapy*. Ulrich, H. Ed.; Springer-Verlag Inc.: San Diego, **2009**; Chapter 16; pp. 255-270.
- [8] Jäderstad, J.; Jäderstad, L.M.; Li, J.; Chintawar, S.; Salto, C.; Pandolfo, M.; Ourednik, V.; Teng, Y.D.; Sidman, R.L.; Arenas, E.; Snyder, E.Y.; Herlenius, E. Communication via gap junctions underlies early functional and beneficial interactions between grafted neural stem cells and the host. *Proc. Natl. Acad. Sci. USA*, **2010**, 107(11), 5184-5189.
- [9] Rosario, C.M.; Yandava, B.D.; Kosaras, B.; Zurakowski, D.; Sidman, R.L.; Snyder, E.Y. Differentiation of engrafted multipotent neural progenitors towards replacement of missing granule neurons in meander tail cerebellum may help determine the locus of mutant gene action. *Development*, **1997**, 124(21), 4213-4224.
- [10] Auerbach, J.M.; Eiden, M.V.; McKay, R.D. Transplanted CNS stem cells form functional synapses *in vivo*. *Eur. J. Neurosci.*, **2000**, 12(5), 1696-1704.
- [11] Chung, S.; Shin, B.S.; Hwang, M.; Lardaro, T.; Kang, U.J.; Isaacson, O.; Kim, K.S. Neural precursors derived from embryonic stem cells, but not those from fetal ventral mesencephalon, maintain the potential to differentiate into dopaminergic neurons after expansion *in vitro*. *Stem Cells*, **2006**, 24(6), 1583-1593.
- [12] Yandava, B.D.; Billingham, L.L.; Snyder, E.Y. "Global" cell replacement is feasible via neural stem Cell Transplantation: evidence from the dysmyelinated shiverer mouse brain. *Proc. Natl. Acad. Sci. USA*, **1999**, 96(12), 7029-7034.
- [13] Imitola, J.; Raddassi, K.; Park, K.L.; Mueller, F.J.; Nieto, M.; Teng, Y.D.; Frenkel, D.; Li, J.; Sidman, R.L.; Walsh, C.A.; Snyder, E.Y.; Khoury, S.J. Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. *Proc. Natl. Acad. Sci. USA*, **2004**, 101(52), 18117-18122.

- [14] Redmond, D.E., Jr.; Bjugstad, K.B.; Teng, Y.D.; Ourednik, V.; Ourednik, J.; Wakeman, D.R.; Parsons, X.H.; Gonzalez, R.; Blanchard, B.C.; Kim, S.U.; Gu, Z.; Lipton, S.A.; Markakis, E.A.; Roth, R.H.; Elsworth, J.D.; Sladek, J.R., Jr.; Sidman, R.L.; Snyder, E.Y. Behavioral improvement in a primate Parkinson's model is associated with multiple homeostatic effects of human neural stem cells. *Proc. Natl. Acad. Sci. USA*, **2007**, *104*(29), 12175-12180.
- [15] Behrstock, S.; Ebert, A.D.; Klein, S.; Schmitt, M.; Moore, J.M.; Svendsen, C.N. Lesion-induced increase in survival and migration of human neural progenitor cells releasing GDNF. *Cell Transplant.*, **2008**, *17*(7), 753-762.
- [16] Aboody, K.S.; Brown, A.; Rainov, N.G.; Bower, K.A.; Liu, S.; Yang, W.; Small, J.E.; Herrlinger, U.; Ourednik, V.; Black, P.M.; Breakefield, X.O.; Snyder, E.Y. Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*(23), 12846-12851.
- [17] Park, K.I.; Teng, Y.D.; Snyder, E.Y. The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. *Nat. Biotechnol.*, **2002**, *20*(11), 1111-1117.
- [18] Ma, D.K.; Ming, G.L.; Song, H. Glial influences on neural stem cell development: cellular niches for adult neurogenesis. *Curr. Opin. Neurobiol.*, **2005**, *15*(5), 514-520.
- [19] Cao, Q.L.; Zhang, Y.P.; Howard, R.M.; Walters, W.M.; Tsoulfas, P.; Whittemore, S.R. Pluripotent stem cells engrafted into the normal or lesioned adult rat spinal cord are restricted to a glial lineage. *Exp. Neurol.*, **2001**, *167*(1), 48-58.
- [20] Teng, Y.D.; Lavik, E.B.; Qu, X.; Park, K.I.; Ourednik, J.; Zurakowski, D.; Langer, R.; Snyder, E.Y. Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*(5), 3024-3029.
- [21] Pluchino, S.; Zanotti, L.; Rossi, B.; Brambilla, E.; Ottoboni, L.; Salani, G.; Martinello, M.; Cattalini, A.; Bergami, A.; Furlan, R.; Comi, G.; Constantin, G.; Martino, G. Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. *Nature*, **2005**, *436*(7048), 266-271.
- [22] Gersh, B.J.; Simari, R.D.; Behfar, A.; Terzic, C.M.; Terzic, A. Cardiac cell repair therapy: a clinical perspective. *Mayo Clin. Proc.*, **2009**, *84*(10), 876-892.
- [23] Redmond, D.E., Jr.; Elsworth, J.D.; Roth, R.H.; Leranthe, C.; Collier, T.J.; Blanchard, B.; Bjugstad, K.B.; Samulski, R.J.; Aebischer, P.; Sladek, J.R., Jr. Embryonic substantia nigra grafts in the mesencephalon send neurites to the host striatum in non-human primate after overexpression of GDNF. *J. Comp. Neurol.*, **2009**, *515*(1), 31-40.
- [24] Mocchiatti, I.; Bachis, A.; Masliah, E. Chemokine receptors and neurotrophic factors: potential therapy against aids dementia? *J. Neurosci. Res.*, **2008**, *86*(2), 243-255.
- [25] Ventura, C.; Cavallini, C.; Bianchi, F.; Cantoni, S. Stem cells and cardiovascular repair: a role for natural and synthetic molecules harboring differentiating and paracrine logics. *Cardiovasc. Hematol. Agents Med. Chem.*, **2008**, *6*(1), 60-68.
- [26] Kaplan, D.R.; Miller, F.D. Neurotrophin signal transduction in the nervous system. *Curr. Opin. Neurobiol.*, **2000**, *10*(3), 381-391.
- [27] Aicardi, G.; Argilli, E.; Cappello, S.; Santi, S.; Riccio, M.; Thoenen, H.; Canossa, M. Induction of long-term potentiation and depression is reflected by corresponding changes in secretion of endogenous brain-derived neurotrophic factor. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*(44), 15788-15792.
- [28] Tuszynski, M.H. Neurotrophic factors. In: *CNS Regeneration: Basic Science and Clinical Advances*. Tuszynski, J.H. Ed.; Academic Press: San Diego, **1999**; pp 109-158.
- [29] Tuszynski, M.H. Nerve growth factor gene delivery: animal models to clinical trials. *Dev. Neurobiol.*, **2007**, *67*(9), 1204-1215.
- [30] Bibel, M.; Barde, Y.A. Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system. *Genes Dev.*, **2000**, *14*(23), 2919-2937.
- [31] Hapner, S.J.; Boeshore, K.L.; Large, T.H.; Lefcort, F. Neural differentiation promoted by truncated trkC receptors in collaboration with p75(NTR). *Dev. Biol.*, **1998**, *201*(1), 90-100.
- [32] Guo, J.S.; Zeng, Y.S.; Li, H.B.; Huang, W.L.; Liu, R.Y.; Li, X.B.; Ding, Y.; Wu, L.Z.; Cai, D.Z. Cotransplant of neural stem cells and NT-3 gene modified Schwann cells promote the recovery of transected spinal cord injury. *Spinal Cord*, **2007**, *45*(1), 15-24.
- [33] Wang, J.M.; Zeng, Y.S.; Liu, R.Y.; Huang, W.L.; Xiong, Y.; Wang, Y.H.; Chen, S.J.; Teng, Y.D. Recombinant adenovirus vector-mediated functional expression of neurotrophin-3 receptor (TrkC) in neural stem cells. *Exp. Neurol.*, **2007**, *203*(1), 123-127.
- [34] Castellanos, D.A.; Tsoulfas, P.; Frydel, B.R.; Gajavelli, S.; Bes, J.C.; Sagen, J. TrkC overexpression enhances survival and migration of neural stem Cell Transplants in the rat spinal cord. *Cell Transplant.*, **2002**, *11*(3), 297-307.
- [35] Bartkowska, K.; Paquin, A.; Gauthier, A.S.; Kaplan, D.R.; Miller, F.D. Trk signaling regulates neural precursor cell proliferation and differentiation during cortical development. *Development*, **2007**, *134*(24), 4369-4380.
- [36] Barker, P.A.; Lomen-Hoerth, C.; Gensch, E.M.; Meakin, S.O.; Glass, D.J.; Shooter, E.M. Tissue-specific alternative splicing generates two isoforms of the trkA receptor. *J. Biol. Chem.*, **1993**, *268*(20), 15150-15157.
- [37] Tacconelli, A.; Farina, A.R.; Cappabianca, L.; Desantis, G.; Tessitore, A.; Vetuschi, A.; Sferra, R.; Rucci, N.; Argenti, B.; Screpanti, I.; Gulino, A.; Mackay, A.R. TrkA alternative splicing: a regulated tumor-promoting switch in human neuroblastoma. *Cancer Cell*, **2004**, *6*(4), 347-360.
- [38] Narumiya, S.; Ohno, M.; Tanaka, N.; Yamano, T.; Shimada, M. Enhanced expression of full-length TrkB receptors in young rat brain with hypoxic/ischemic injury. *Brain Res.*, **1998**, *797*(2), 278-286.
- [39] Fryer, R.H.; Kaplan, D.R.; Kromer, L.F. Truncated trkB receptors on nonneuronal cells inhibit BDNF-induced neurite outgrowth *in vitro*. *Exp. Neurol.*, **1997**, *148*(2), 616-627.
- [40] Bartkowska, K.; Paquin, A.; Gauthier, A.S.; Kaplan, D.R.; Miller, F.D. Trk signaling regulates neural precursor cell proliferation and differentiation during cortical development. *Development*, **2007**, *134*(24), 4369-4380.
- [41] Hempstead, B.L. The many faces of p75NTR. *Curr. Opin. Neurobiol.*, **2002**, *12*(3), 260-267.
- [42] Islam, O.; Loo, T.X.; Heese, K. Brain-derived neurotrophic factor (BDNF) has proliferative effects on neural stem cells through the truncated TRK-B receptor, MAP kinase, AKT, and STAT-3 signaling pathways. *Curr. Neurovasc. Res.*, **2009**, *6*(1), 42-53.
- [43] Naumann, T.; Casademunt, E.; Hollerbach, E.; Hofmann, J.; Dechant, G.; Frotscher, M.; Barde, Y.A. Complete deletion of the neurotrophin receptor p75NTR leads to long-lasting increases in the number of basal forebrain cholinergic neurons. *J. Neurosci.*, **2002**, *22*(7), 2409-2418.
- [44] Troy, C.M.; Friedman, J.E.; Friedman, W.J. Mechanisms of p75-mediated death of hippocampal neurons. Role of caspases. *J. Biol. Chem.*, **2002**, *277*(37), 34295-34302.
- [45] Moscatelli, I.; Pierantozzi, E.; Camaioni, A.; Siracusa, G.; Campagnolo, L. p75 neurotrophin receptor is involved in proliferation of undifferentiated mouse embryonic stem cells. *Exp. Cell Res.*, **2009**, *315*(18), 3220-3232.
- [46] Zhang, W.; Zeng, Y.S.; Wang, J.M.; Ding, Y.; Li, Y.; Wu, W. Neurotrophin-3 improves retinoic acid-induced neural differentiation of skin-derived precursors through a p75NTR-dependent signaling pathway. *Neurosci. Res.*, **2009**, *64*(2), 170-176.
- [47] Morris, Z.S.; McClatchey, A.I. The neurofibroma cell of origin: SKPs expand the playing field. *Cell Stem Cell*, **2009**, *4*(5), 371-372.
- [48] Reier, P.J. Cellular transplantation strategies for spinal cord injury and translational neurobiology. *NeuroRx*, **2004**, *1*(4), 424-451.
- [49] Fitch, M.T.; Silver, J. CNS injury, glial scars, and inflammation: Inhibitory extracellular matrices and regeneration failure. *Exp. Neurol.*, **2008**, *209*(2), 294-301.
- [50] Terenghi, G. Peripheral nerve regeneration and neurotrophic factors. *J. Anat.*, **1999**, *194* (Pt 1), 1-14.
- [51] Boyd, J.G.; Gordon, T. Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. *Mol. Neurobiol.*, **2003**, *27*(3), 277-324.
- [52] Silver, J.; Miller, J.H. Regeneration beyond the glial scar. *Nat. Rev. Neurosci.*, **2004**, *5*(2), 146-156.
- [53] Fawcett, J.W. Overcoming inhibition in the damaged spinal cord. *J. Neurotrauma*, **2006**, *23*(3-4), 371-383.
- [54] Neumann, S.; Woolf, C.J. Regeneration of dorsal column fibers into and beyond the lesion site following adult spinal cord injury. *Neuron*, **1999**, *23*(1), 83-91.

- [55] Seijffers, R.; Mills, C.D.; Woolf, C.J. ATF3 increases the intrinsic growth state of DRG neurons to enhance peripheral nerve regeneration. *J. Neurosci.*, **2007**, 27(30), 7911-7920.
- [56] Lakatos, A.; Franklin, R.J. Transplant mediated repair of the central nervous system: an imminent solution? *Curr. Opin. Neurol.*, **2002**, 15(6), 701-705.
- [57] Tuszynski, M.H. Growth-factor gene therapy for neurodegenerative disorders. *Lancet Neurol.*, **2002**, 1(1), 51-57.
- [58] Qiu, J.; Cai, D.; Dai, H.; McAtee, M.; Hoffman, P.N.; Bregman, B.S.; Filbin, M.T. Spinal axon regeneration induced by elevation of cyclic AMP. *Neuron*, **2002**, 34(6), 895-903.
- [59] Novikova, L.N.; Novikov, L.N.; Kellerth, J.O. Biopolymers and biodegradable smart implants for tissue regeneration after spinal cord injury. *Curr. Opin. Neurol.*, **2003**, 16(6), 711-715.
- [60] Rossi, F.; Gianola, S.; Corvetto, L. Regulation of intrinsic neuronal properties for axon growth and regeneration. *Prog. Neurobiol.*, **2007**, 81(1), 1-28.
- [61] Lu, P.; Yang, H.; Jones, L.L.; Filbin, M.T.; Tuszynski, M.H. Combinatorial therapy with neurotrophins and cAMP promotes axonal regeneration beyond sites of spinal cord injury. *J. Neurosci.*, **2004**, 24(28), 6402-6409.
- [62] Nikulina, E.; Tidwell, J.L.; Dai, H.N.; Bregman, B.S.; Filbin, M.T. The phosphodiesterase inhibitor rolipram delivered after a spinal cord lesion promotes axonal regeneration and functional recovery. *Proc. Natl. Acad. Sci. USA*, **2004**, 101(23), 8786-8790.
- [63] Pearce, D.D.; Pereira, F.C.; Marcillo, A.E.; Bates, M.L.; Berrocal, Y.A.; Filbin, M.T.; Bunge, M.B. cAMP and Schwann cells promote axonal growth and functional recovery after spinal cord injury. *Nat. Med.*, **2004**, 10(6), 610-616.
- [64] Kobayashi, N.R.; Fan, D.P.; Giehl, K.M.; Bedard, A.M.; Wiegand, S.J.; Tetzlaff, W. BDNF and NT-4/5 prevent atrophy of rat rubrospinal neurons after cervical axotomy, stimulate GAP-43 and α -tubulin mRNA expression, and promote axonal regeneration. *J. Neurosci.*, **1997**, 17(24), 9583-9595.
- [65] Liu, Y.; Kim, D.; Himes, B.T.; Chow, S.Y.; Schallert, T.; Murray, M.; Tessler, A.; Fischer, I. Transplants of fibroblasts genetically modified to express BDNF promote regeneration of adult rat rubrospinal axons and recovery of forelimb function. *J. Neurosci.*, **1999**, 19(11), 4370-4387.
- [66] Jin, Y.; Fischer, I.; Tessler, A.; Houle, J.D. Transplants of fibroblasts genetically modified to express BDNF promote axonal regeneration from supraspinal neurons following chronic spinal cord injury. *Exp. Neurol.*, **2002**, 177(1), 265-275.
- [67] Kwon, B.K.; Liu, J.; Messerer, C.; Kobayashi, N.R.; McGraw, J.; Oschepok, L.; Tetzlaff, W. Survival and regeneration of rubrospinal neurons 1 year after spinal cord injury. *Proc. Natl. Acad. Sci. USA*, **2002**, 99(5), 3246-3251.
- [68] Lu, P.; Blesch, A.; Tuszynski, M.H. Neurotrophism without neurotrophism: BDNF promotes survival but not growth of lesioned corticospinal neurons. *J. Comp. Neurol.*, **2001**, 436(4), 456-470.
- [69] Schnell, L.; Schneider, R.; Kolbeck, R.; Barde, Y.A.; Schwab, M.E. Neurotrophin-3 enhances sprouting of corticospinal tract during development and after adult spinal cord lesion. *Nature*, **1994**, 367(6459), 170-173.
- [70] Grill, R.; Murai, K.; Blesch, A.; Gage, F.H.; Tuszynski, M.H. Cellular delivery of neurotrophin-3 promotes corticospinal axonal growth and partial functional recovery after spinal cord injury. *J. Neurosci.*, **1997**, 17(14), 5560-5572.
- [71] Kadoya, K.; Tsukada, S.; Lu, P.; Coppola, G.; Geschwind, D.; Filbin, M.T.; Blesch, A.; Tuszynski, M.H. Combined intrinsic and extrinsic neuronal mechanisms facilitate bridging axonal regeneration one year after spinal cord injury. *Neuron*, **2009**, 64(2), 165-172.
- [72] Hendriks, W.T.; Ruitenbergh, M.J.; Blits, B.; Boer, G.J.; Verhaagen, J. Viral vector-mediated gene transfer of neurotrophins to promote regeneration of the injured spinal cord. *Prog. Brain Res.*, **2004**, 146, 451-476.
- [73] Ying, Z.; Roy, R.R.; Zhong, H.; Zdunowski, S.; Edgerton, V.R.; Gomez-Pinilla, F. BDNF-exercise interactions in the recovery of symmetrical stepping after a cervical hemisection in rats. *Neuroscience*, **2008**, 155(4), 1070-1078.
- [74] Konya, D.; Liao, W.L.; Choi, H.; Yu, D.; Woodard, M.C.; Newton, K.M.; King, A.M.; Pamir, N.M.; Black, P.M.; Frontera, W.R.; Sabharwal, S.; Teng, Y.D. Functional recovery in T13-L1 hemisection rats resulting from peripheral nerve rerouting: role of central neuroplasticity. *Regen. Med.*, **2008**, 3(3), 309-327.
- [75] Foret, A.; Quertainmont, R.; Botman, O.; Bouhy, D.; Amabili, P.; Brook, G.; Schoenen, J.; Franzen, R. Stem cells in the adult rat spinal cord: plasticity after injury and treadmill training exercise. *J. Neurochem.*, **2010**, 112(3), 762-772.
- [76] Behrman, A.L.; Bowden, M.G.; Nair, P.M. Neuroplasticity after spinal cord injury and training: an emerging paradigm shift in rehabilitation and walking recovery. *Phys. Ther.*, **2006**, 86(10), 1406-1425.
- [77] Mocchetti, I.; Brown, M. Targeting neurotrophin receptors in the central nervous system. *CNS Neurol. Disord. Drug Targets*, **2008**, 7(1), 71-82.
- [78] Adrian, E.K.; Jr.; Walker, B.E. Incorporation of thymidine-H3 by cells in normal and injured mouse spinal cord. *J. Neuropathol. Exp. Neurol.*, **1962**, 21, 597-609.
- [79] Weiss, S.; Dunne, C.; Hewson, J.; Wohl, C.; Wheatley, M.; Peterson, A.C.; Reynolds, B.A. Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. *J. Neurosci.*, **1996**, 16(23), 7599-7609.
- [80] Tramontin, A.D.; Garcia-Verdugo, J.M.; Lim, D.A.; Alvarez-Buylla, A. Postnatal development of radial glia and the ventricular zone (VZ): a continuum of the neural stem cell compartment. *Cereb. Cortex*, **2003**, 13(6), 580-587.
- [81] Shihabuddin, L.S.; Horner, P.J.; Ray, J.; Gage, F.H. Adult spinal cord stem cells generate neurons after transplantation in the adult dentate gyrus. *J. Neurosci.*, **2000**, 20(23), 8727-8735.
- [82] Horner, P.J.; Power, A.E.; Kempermann, G.; Kuhn, H.G.; Palmer, T.D.; Winkler, J.; Thal, L.J.; Gage, F.H. Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. *J. Neurosci.*, **2000**, 20(6), 2218-2228.
- [83] Song, H.J.; Stevens, C.F.; Gage, F.H. Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. *Nat. Neurosci.*, **2002**, 5(5), 438-445.
- [84] Frisen, J.; Johansson, C.B.; Torok, C.; Risling, M.; Lendahl, U. Rapid, widespread, and longlasting induction of nestin contributes to the generation of glial scar tissue after CNS injury. *J. Cell Biol.*, **1995**, 131(2), 453-464.
- [85] Shihabuddin, L.S.; Palmer, T.D.; Gage, F.H. The search for neural progenitor cells: prospects for the therapy of neurodegenerative disease. *Mol. Med. Today*, **1999**, 5(11), 474-480.
- [86] Yamamoto, S.; Yamamoto, N.; Kitamura, T.; Nakamura, K.; Nakafuku, M. Proliferation of parenchymal neural progenitors in response to injury in the adult rat spinal cord. *Exp. Neurol.*, **2001**, 172(1), 115-127.
- [87] Matthews, M.A.; St Onge, M.F.; Faciane, C.L. An electron microscopic analysis of abnormal ependymal cell proliferation and envelopment of sprouting axons following spinal cord transection in the rat. *Acta Neuropathol.*, **1979**, 45(1), 27-36.
- [88] Tripathi, R.B.; McTigue, D.M. Chronically increased ciliary neurotrophic factor and fibroblast growth factor-2 expression after spinal contusion in rats. *J. Comp. Neurol.*, **2008**, 510(2), 129-144.
- [89] Takahashi, M.; Arai, Y.; Kurosawa, H.; Sueyoshi, N.; Shirai, S. Ependymal cell reactions in spinal cord segments after compression injury in adult rat. *J. Neuropathol. Exp. Neurol.*, **2003**, 62(2), 185-194.
- [90] McTigue, D.M.; Horner, P.J.; Stokes, B.T.; Gage, F.H. Neurotrophin-3 and brain-derived neurotrophic factor induce oligodendrocyte proliferation and myelination of regenerating axons in the contused adult rat spinal cord. *J. Neurosci.*, **1998**, 18(14), 5354-5365.
- [91] Mothe, A.J.; Tator, C.H. Proliferation, migration, and differentiation of endogenous ependymal region stem/progenitor cells following minimal spinal cord injury in the adult rat. *Neuroscience*, **2005**, 131(1), 177-187.
- [92] Kempermann, G.; Kuhn, H.G.; Gage, F.H. More hippocampal neurons in adult mice living in an enriched environment. *Nature*, **1997**, 386(6624), 493-495.
- [93] Teng, Y.D.; Liao, W.L.; Choi, H.; Konya, D.; Sabharwal, S.; Langer, R.; Sidman, R.L.; Snyder, E.Y.; Frontera, W.R. Physical activity-mediated functional recovery after spinal cord injury: potential roles of neural stem cells. *Regen. Med.*, **2006**, 1(6), 763-776.
- [94] Gomez-Pinilla, F.; Ying, Z.; Roy, R.R.; Molteni, R.; Edgerton, V.R. Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity. *J. Neurophysiol.*, **2002**, 88(5), 2187-2195.
- [95] Jakeman, L.B.; Wei, P.; Guan, Z.; Stokes, B.T. Brain-derived neurotrophic factor stimulates hindlimb stepping and sprouting of cho-

- linergic fibers after spinal cord injury. *Exp. Neurol.*, **1998**, *154*(1), 170-184.
- [96] Ying, Z.; Roy, R.R.; Edgerton, V.R.; Gomez-Pinilla, F. Voluntary exercise increases neurotrophin-3 and its receptor TrkC in the spinal cord. *Brain Res.*, **2003**, *987*(1), 93-99.
- [97] Danzer, S.C.; He, X.; McNamara, J.O. Ontogeny of seizure-induced increases in BDNF immunoreactivity and TrkB receptor activation in rat hippocampus. *Hippocampus*, **2004**, *14*(3), 345-355.
- [98] Osaka, M.; Honmou, O.; Murakami, T.; Nonaka, T.; Houkin, K.; Hamada, H.; Kocsis, J.D. Intravenous administration of mesenchymal stem cells derived from bone marrow after contusive spinal cord injury improves functional outcome. *Brain Res.*, **2010**, *1343*, 226-235.
- [99] van Praag, H.; Kempermann, G.; Gage, F.H. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat. Neurosci.*, **1999**, *2*(3), 266-270.
- [100] van Praag, H.; Christie, B.R.; Sejnowski, T.J.; Gage, F.H. Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc. Natl. Acad. Sci. USA*, **1999**, *96*(23), 13427-13431.
- [101] Vaynman, S.; Gomez-Pinilla, F. License to run: exercise impacts functional plasticity in the intact and injured central nervous system by using neurotrophins. *Neurorehabil. Neural Repair*, **2005**, *19*(4), 283-295.
- [102] Krityakiarana, W.; Espinosa-Jeffrey, A.; Ghiani, C.A.; Zhao, P.M.; Topaladjikian, N.; Gomez-Pinilla, F.; Yamaguchi, M.; Kotchabhakdi, N.; de Vellis, J. Voluntary exercise increases oligodendrogenesis in spinal cord. *Int. J. Neurosci.*, **2010**, *120*(4), 280-290.
- [103] Snyder, E.Y.; Taylor, R.M.; Wolfe, J.H. Neural progenitor cell engraftment corrects lysosomal storage throughout the MPS VII mouse brain. *Nature*, **1995**, *374*(6520), 367-370.
- [104] Imitola, J.; Park, K.I.; Teng, Y.D.; Nisim, S.; Lachyankar, M.; Ourednik, J.; Mueller, F.J.; Yiou, R.; Atala, A.; Sidman, R.L.; Tuszynski, M.; Khoury, S.J.; Snyder, E.Y. Stem cells: cross-talk and developmental programs. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **2004**, *359*(1445), 823-837.
- [105] Flax, J.D.; Aurora, S.; Yang, C.; Simonin, C.; Wills, A.M.; Billingham, L.L.; Jendoubi, M.; Sidman, R.L.; Wolfe, J.H.; Kim, S.U.; Snyder, E.Y. Engraftable human neural stem cells respond to developmental cues, replace neurons, and express foreign genes. *Nat. Biotechnol.*, **1998**, *16*(11), 1033-1039.
- [106] Hill, C.E.; Guller, Y.; Raffa, S.J.; Hurtado, A.; Bunge, M. A calpain inhibitor enhances the survival of Schwann cells *in vitro* and after transplantation into the injured spinal cord. *J. Neurotrauma*, **2010**, *27*(9), 1685-1695.
- [107] Yu, D.; Neeley, W.L.; Pritchard, C.D.; Slotkin, J.R.; Woodard, E.J.; Langer, R.; Teng, Y.D. Blockade of peroxynitrite-induced neural stem cell death in the acutely injured spinal cord by drug-releasing polymer. *Stem Cells*, **2009**, *27*(5), 1212-1222.
- [108] Hedlund, E.; Hefferan, M.P.; Marsala, M.; Isacson, O. Cell therapy and stem cells in animal models of motor neuron disorders. *Eur. J. Neurosci.*, **2007**, *26*(7), 1721-1737.
- [109] Suzuki, M.; Svendsen, C.N. Combining growth factor and stem cell therapy for amyotrophic lateral sclerosis. *Trends Neurosci.*, **2008**, *31*(4), 192-198.
- [110] Lu, P.; Jones, L.L.; Snyder, E.Y.; Tuszynski, M.H. Neural stem cells constitutively secrete neurotrophic factors and promote extensive host axonal growth after spinal cord injury. *Exp. Neurol.*, **2003**, *181*(2), 115-129.
- [111] Levi-Montalcini, R. The nerve growth factor 35 years later. *Science*, **1987**, *237*(4819), 1154-1162.
- [112] Levi-Montalcini, R.; Hamburger, V. Selective growth stimulating effects of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo. *J. Exp. Zool.*, **1951**, *116*(2), 321-361.
- [113] Fischer, W.; Victorin, K.; Bjorklund, A.; Williams, L.R.; Varon, S.; Gage, F.H. Amelioration of cholinergic neuron atrophy and spatial memory impairment in aged rats by nerve growth factor. *Nature*, **1987**, *329*(6134), 65-68.
- [114] Markowska, A.L.; Koliatsos, V.E.; Breckler, S.J.; Price, D.L.; Olton, D.S. Human nerve growth factor improves spatial memory in aged but not in young rats. *J. Neurosci.*, **1994**, *14*(8), 4815-4824.
- [115] Bezprozvanny, I.; Mattson, M.P. Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. *Trends Neurosci.*, **2008**, *31*(9), 454-463.
- [116] Koliatsos, V.E.; Clatterbuck, R.E.; Nauta, H.J.; Knusel, B.; Burton, L.E.; Hefti, F.F.; Mobley, W.C.; Price, D.L. Human nerve growth factor prevents degeneration of basal forebrain cholinergic neurons in primates. *Ann. Neurol.*, **1991**, *30*(6), 831-840.
- [117] Emerich, D.F.; Winn, S.R.; Harper, J.; Hammang, J.P.; Baetge, E.E.; Kordower, J.H. Implants of polymer-encapsulated human NGF-secreting cells in the nonhuman primate: rescue and sprouting of degenerating cholinergic basal forebrain neurons. *J. Comp. Neurol.*, **1994**, *349*(1), 148-164.
- [118] Kordower, J.H.; Winn, S.R.; Liu, Y.T.; Mufson, E.J.; Sladek, J.R., Jr.; Hammang, J.P.; Baetge, E.E.; Emerich, D.F. The aged monkey basal forebrain: rescue and sprouting of axotomized basal forebrain neurons after grafts of encapsulated cells secreting human nerve growth factor. *Proc. Natl. Acad. Sci. USA*, **1994**, *91*(23), 10898-10902.
- [119] Holtzman, D.M.; Li, Y.; Parada, L.F.; Kinsman, S.; Chen, C.K.; Valletta, J.S.; Zhou, J.; Long, J.B.; Mobley, W.C. p140trk mRNA marks NGF-responsive forebrain neurons: evidence that trk gene expression is induced by NGF. *Neuron*, **1992**, *9*(3), 465-478.
- [120] Tuszynski, M.H.; Gage, F.H. Bridging grafts and transient nerve growth factor infusions promote long-term central nervous system neuronal rescue and partial functional recovery. *Proc. Natl. Acad. Sci. USA*, **1995**, *92*(10), 4621-4625.
- [121] Cooper, J.D.; Salehi, A.; Delcroix, J.D.; Howe, C.L.; Belichenko, P.V.; Chua-Couzens, J.; Kilbridge, J.F.; Carlson, E.J.; Epstein, C.J.; Mobley, W.C. Failed retrograde transport of NGF in a mouse model of Down's syndrome: reversal of cholinergic neurodegenerative phenotypes following NGF infusion. *Proc. Natl. Acad. Sci. USA*, **2001**, *98*(18), 10439-10444.
- [122] Tuszynski, M.H.; Roberts, J.; Senut, M.C.; U, H.S.; Gage, F.H. Gene therapy in the adult primate brain: intraparenchymal grafts of cells genetically modified to produce nerve growth factor prevent cholinergic neuronal degeneration. *Gene Ther.*, **1996**, *3*(4), 305-314.
- [123] Perry, E.K.; Curtis, M.; Dick, D.J.; Candy, J.M.; Atack, J.R.; Bloxham, C.A.; Blessed, G.; Fairbairn, A.; Tomlinson, B.E.; Perry, R.H. Cholinergic correlates of cognitive impairment in Parkinson's disease: comparisons with Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry*, **1985**, *48*(5), 413-421.
- [124] Perry, E.K.; Tomlinson, B.E.; Blessed, G.; Bergmann, K.; Gibson, P.H.; Perry, R.H. Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *Br. Med. J.*, **1978**, *2*(6150), 1457-1459.
- [125] Candy, J.M.; Perry, R.H.; Perry, E.K.; Irving, D.; Blessed, G.; Fairbairn, A.F.; Tomlinson, B.E. Pathological changes in the nucleus of Meynert in Alzheimer's and Parkinson's diseases. *J. Neurol. Sci.*, **1983**, *59*(2), 277-289.
- [126] Masliah, E.; Terry, R.D.; Alford, M.; DeTeresa, R.; Hansen, L.A. Cortical and subcortical patterns of synaptophysin-like immunoreactivity in Alzheimer's disease. *Am. J. Pathol.*, **1991**, *138*(1), 235-246.
- [127] Mufson, E.J.; Ikonomic, M.D.; Styren, S.D.; Counts, S.E.; Wu, J.; Leurgans, S.; Bennett, D.A.; Cochran, E.J.; DeKosky, S.T. Preservation of brain nerve growth factor in mild cognitive impairment and Alzheimer disease. *Arch. Neurol.*, **2003**, *60*(8), 1143-1148.
- [128] Korsching, S.; Auburger, G.; Heumann, R.; Scott, J.; Thoenen, H. Levels of nerve growth factor and its mRNA in the central nervous system of the rat correlate with cholinergic innervation. *EMBO J.*, **1985**, *4*(6), 1389-1393.
- [129] Whittemore, S.R.; Ebendal, T.; Larkfors, L.; Olson, L.; Seiger, A.; Stromberg, I.; Persson, H. Development and regional expression of beta nerve growth factor messenger RNA and protein in the rat central nervous system. *Proc. Natl. Acad. Sci. USA*, **1986**, *83*(3), 817-821.
- [130] Savaskan, E.; Muller-Spahn, F.; Olivieri, G.; Bruttel, S.; Otten, U.; Rosenberg, C.; Hulette, C.; Hock, C. Alterations in trk A, trk B and trk C receptor immunoreactivities in parietal cortex and cerebellum in Alzheimer's disease. *Eur. Neurol.*, **2000**, *44*(3), 172-180.
- [131] Chu, Y.; Cochran, E.J.; Bennett, D.A.; Mufson, E.J.; Kordower, J.H. Down-regulation of trkA mRNA within nucleus basalis neurons in individuals with mild cognitive impairment and Alzheimer's disease. *J. Comp. Neurol.*, **2001**, *437*(3), 296-307.
- [132] Mufson, E.J.; Ma, S.Y.; Dills, J.; Cochran, E.J.; Leurgans, S.; Wu, J.; Bennett, D.A.; Jaffar, S.; Gilmor, M.L.; Levey, A.I.; Kordower, J.H. Loss of basal forebrain P75(NTR) immunoreactivity in sub-

- jects with mild cognitive impairment and Alzheimer's disease. *J. Comp. Neurol.*, **2002**, *443*(2), 136-153.
- [133] Salehi, A.; Delcroix, J.D.; Belichenko, P.V.; Zhan, K.; Wu, C.; Valletta, J.S.; Takimoto-Kimura, R.; Kleschevnikov, A.M.; Sambamurti, K.; Chung, P.P.; Xia, W.; Villar, A.; Campbell, W.A.; Kulnane, L.S.; Nixon, R.A.; Lamb, B.T.; Epstein, C.J.; Stokin, G.B.; Goldstein, L.S.; Mobley, W.C. Increased App expression in a mouse model of Down's syndrome disrupts NGF transport and causes cholinergic neuron degeneration. *Neuron*, **2006**, *51*(1), 29-42.
- [134] Williams, L.R. Hypophagia is induced by intracerebroventricular administration of nerve growth factor. *Exp. Neurol.*, **1991**, *113*(1), 31-37.
- [135] Isaacson, L.G.; Saffran, B.N.; Crutcher, K.A. Intracerebral NGF infusion induces hyperinnervation of cerebral blood vessels. *Neurobiol. Aging*, **1990**, *11*(1), 51-55.
- [136] Emmett, C.J.; Stewart, G.R.; Johnson, R.M.; Aswani, S.P.; Chan, R.L.; Jakeman, L.B. Distribution of radioiodinated recombinant human nerve growth factor in primate brain following intracerebroventricular infusion. *Exp. Neurol.*, **1996**, *140*(2), 151-160.
- [137] Winkler, J.; Ramirez, G.A.; Kuhn, H.G.; Peterson, D.A.; Day-Lollini, P.A.; Stewart, G.R.; Tuszyński, M.H.; Gage, F.H.; Thal, L.J. Reversible Schwann cell hyperplasia and sprouting of sensory and sympathetic neurites after intraventricular administration of nerve growth factor. *Ann. Neurol.*, **1997**, *41*(1), 82-93.
- [138] Eriksson-Jonhagen, M.; Nordberg, A.; Amberla, K.; Backman, L.; Ebendal, T.; Meyerson, B.; Olson, L.; Seiger, Shigeta, M.; Theodorsson, E.; Viitanen, M.; Winblad, B.; Wahlund, L.O. Intracerebroventricular infusion of nerve growth factor in three patients with Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.*, **1998**, *9*(5), 246-257.
- [139] Kordower, J.H.; Palfi, S.; Chen, E.Y.; Ma, S.Y.; Sendera, T.; Cochran, E.J.; Mufson, E.J.; Penn, R.; Goetz, C.G.; Comella, C.D. Clinicopathological findings following intraventricular glial-derived neurotrophic factor treatment in a patient with Parkinson's disease. *Ann. Neurol.*, **1999**, *46*(3), 419-424.
- [140] Nutt, J.G.; Burchiel, K.J.; Comella, C.L.; Jankovic, J.; Lang, A.E.; Laws, E.R., Jr.; Lozano, A.M.; Penn, R.D.; Simpson, R.K., Jr.; Stacy, M.; Wooten, G.F. Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD. *Neurology*, **2003**, *60*(1), 69-73.
- [141] Cleveland, D.W.; Rothstein, J.D. From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. *Nat. Rev. Neurosci.*, **2001**, *2*(11), 806-819.
- [142] Julien, J.P. Amyotrophic lateral sclerosis: unfolding the toxicity of the misfolded. *Cell*, **2001**, *104*(4), 581-591.
- [143] Doble, A. The pharmacology and mechanism of action of riluzole. *Neurology*, **1996**, *47*(6 Suppl 4), S233-241.
- [144] Peluffo, H.; Estevez, A.; Barbeito, L.; Stutzmann, J.M. Riluzole promotes survival of rat motoneurons *in vitro* by stimulating trophic activity produced by spinal astrocyte monolayers. *Neurosci. Lett.*, **1997**, *228*(3), 207-211.
- [145] Brown, R.H., Jr. Amyotrophic lateral sclerosis: recent insights from genetics and transgenic mice. *Cell*, **1995**, *80*(5), 687-692.
- [146] Andersen, P.M.; Sims, K.B.; Xin, W.W.; Kiely, R.; O'Neill, G.; Ravits, J.; Pioro, E.; Harati, Y.; Brower, R.D.; Levine, J.S.; Heinicke, H.U.; Seltzer, W.; Boss, M.; Brown, R.H., Jr. Sixteen novel mutations in the Cu/Zn superoxide dismutase gene in amyotrophic lateral sclerosis: a decade of discoveries, defects and disputes. *Amyotroph. Latera. Scler. Other Motor Neuron Disord.*, **2003**, *4*(2), 62-73.
- [147] Garbuzova-Davis, S.; Willing, A.E.; Milliken, M.; Saporta, S.; Zigova, T.; Cahill, D.W.; Sanberg, P.R. Positive effect of transplantation of hNT neurons (NTERA 2/D1 cell-line) in a model of familial amyotrophic lateral sclerosis. *Exp. Neurol.*, **2002**, *174*(2), 169-180.
- [148] Corti, S.; Locatelli, F.; Donadoni, C.; Guglieri, M.; Papadimitriou, D.; Strazzer, S.; Del Bo, R.; Comi, G.P. Wild-type bone marrow cells ameliorate the phenotype of SOD1-G93A ALS mice and contribute to CNS, heart and skeletal muscle tissues. *Brain*, **2004**, *127*(Pt 11), 2518-2532.
- [149] Hemendinger, R.; Wang, J.; Malik, S.; Persinski, R.; Copeland, J.; Emerich, D.; Gores, P.; Halberstadt, C.; Rosenfeld, J. Sertoli cells improve survival of motor neurons in SOD1 transgenic mice, a model of amyotrophic lateral sclerosis. *Exp. Neurol.*, **2005**, *196*(2), 235-243.
- [150] Yan, J.; Xu, L.; Welsh, A.M.; Chen, D.; Hazel, T.; Johe, K.; Koliatos, V.E. Combined immunosuppressive agents or CD4 antibodies prolong survival of human neural stem cell grafts and improve disease outcomes in amyotrophic lateral sclerosis transgenic mice. *Stem Cells*, **2006**, *24*(8), 1976-1985.
- [151] Habisch, H.J.; Janowski, M.; Binder, D.; Kuzma-Kozakiewicz, M.; Widmann, A.; Habich, A.; Schwalenstocker, B.; Hermann, A.; Brenner, R.; Lukomska, B.; Domanska-Janik, K.; Ludolph, A.C.; Storch, A. Intrathecal application of neuroectodermally converted stem cells into a mouse model of ALS: limited intraparenchymal migration and survival narrows therapeutic effects. *J. Neural. Transm.*, **2007**, *114*(11), 1395-1406.
- [152] Garbuzova-Davis, S.; Klasko, S.K.; Sanberg, P.R. Intravenous administration of human umbilical cord blood cells in an animal model of MPS III B. *J. Comp. Neurol.*, **2009**, *515*(1), 93-101.
- [153] Svendsen, C.N.; ter Borg, M.G.; Armstrong, R.J.; Rosser, A.E.; Chandran, S.; Ostenfeld, T.; Caldwell, M.A. A new method for the rapid and long term growth of human neural precursor cells. *J. Neurosci. Methods*, **1998**, *85*(2), 141-152.
- [154] Teng Y.D.; Benn S.C.; Kalkanis S.N.; Shefner J.M.; Onario R.C.; Cheng B.; Lachyankar M.B.; Macconi M.; Li J.; Maragakis N.J.; Llado J.; Erkmén K.; Redmond D.E. Jr.; Sidman R.L.; Przedborski S.; Rothstein J.D.; Brown R.H. Jr.; Snyder E.Y. Neural stem cells, acting through multiple mechanisms, extensively prolong life and preserve function in a mouse model of ALS. **2010 to be submitted**.
- [155] Xu, L.; Yan, J.; Chen, D.; Welsh, A.M.; Hazel, T.; Johe, K.; Hatfield, G.; Koliatos, V.E. Human neural stem cell grafts ameliorate motor neuron disease in SOD-1 transgenic rats. *Transplantation*, **2006**, *82*(7), 865-875.
- [156] Klein, S.M.; Behrstock, S.; McHugh, J.; Hoffmann, K.; Wallace, K.; Suzuki, M.; Aebischer, P.; Svendsen, C.N. GDNF delivery using human neural progenitor cells in a rat model of ALS. *Hum. Gene Ther.*, **2005**, *16*(4), 509-521.
- [157] Guillot, S.; Azzouz, M.; Deglon, N.; Zurn, A.; Aebischer, P. Local GDNF expression mediated by lentiviral vector protects facial nerve motoneurons but not spinal motoneurons in SOD1(G93A) transgenic mice. *Neurobiol. Dis.*, **2004**, *16*(1), 139-149.
- [158] Suzuki, M.; McHugh, J.; Tork, C.; Shelley, B.; Klein, S.M.; Aebischer, P.; Svendsen, C.N. GDNF secreting human neural progenitor cells protect dying motor neurons, but not their projection to muscle, in a rat model of familial ALS. *PLoS One*, **2007**, *2*(1), e689.
- [159] Mazzini, L.; Ferrero, I.; Luparello, V.; Rustichelli, D.; Gunetti, M.; Mareschi, K.; Testa, L.; Stecco, A.; Tarletti, R.; Miglioretti, M.; Fava, E.; Nasuelli, N.; Cisari, C.; Massara, M.; Vercelli, R.; Oggioni, G.D.; Carriero, A.; Cantello, R.; Monaco, F.; Fagioli, F. Mesenchymal stem Cell Transplantation in amyotrophic lateral sclerosis: A Phase I clinical trial. *Exp. Neurol.*, **2010**, *223*(1), 229-237.
- [160] Group A-CTS. A double-blind placebo-controlled clinical trial of subcutaneous recombinant human ciliary neurotrophic factor (rhCNTF) in amyotrophic lateral sclerosis. ALS CNTF Treatment Study Group. *Neurology*, **1996**, *46*(5), 1244-1249.
- [161] Group TBS. A controlled trial of recombinant methionyl human BDNF in ALS: The BDNF Study Group (Phase III). *Neurology*, **1999**, *52*(7), 1427-1433.
- [162] Nagai, M.; Re, D.B.; Nagata, T.; Chalazonitis, A.; Jessell, T.M.; Wichterle, H.; Przedborski, S. Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. *Nat. Neurosci.*, **2007**, *10*(5), 615-622.
- [163] Yamanaka, K.; Chun, S.J.; Boillee, S.; Fujimori-Tonou, N.; Yamashita, H.; Gutmann, D.H.; Takahashi, R.; Misawa, H.; Cleveland, D.W. Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. *Nat. Neurosci.*, **2008**, *11*(3), 251-253.
- [164] Takahashi, K.; Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, **2006**, *126*(4), 663-676.
- [165] Pick, M.; Stelzer, Y.; Bar-Nur, O.; Mayshar, Y.; Eden, A.; Benvenisty, N. Clone- and gene-specific aberrations of parental imprinting in human induced pluripotent stem cells. *Stem Cells*, **2009**, *27*(11), 2686-2690.
- [166] Marchetto, M.C.; Yeo, G.W.; Kainohana, O.; Marsala, M.; Gage, F.H.; Muotri, A.R. Transcriptional signature, and memory retention

- tion of human-induced pluripotent stem cells. *PLoS One*, **2009**, 4(9), e7076.
- [167] Ghosh, Z.; Wilson, K.D.; Wu, Y.; Hu, S.; Quertermous, T.; Wu, J.C. Persistent donor cell gene expression among human induced pluripotent stem cells contributes to differences with human embryonic stem cells. *PLoS One*, **2010**, 5(2), e8975.
- [168] Heng, J.C.; Feng, B.; Han, J.; Jiang, J.; Kraus, P.; Ng, J.H.; Orlov, Y.L.; Huss, M.; Yang, L.; Lufkin, T.; Lim, B.; Ng, H.H. The nuclear receptor Nr5a2 can replace Oct4 in the reprogramming of murine somatic cells to pluripotent cells. *Cell Stem Cell*, **2010**, 6(2), 167-174.
- [169] O'Malley, J.; Woltjen, K.; Kaji, K. New strategies to generate induced pluripotent stem cells. *Curr. Opin. Biotechnol.*, **2009**, 20(5), 516-521.
- [170] Huangfu, D.; Osafune, K.; Maehr, R.; Guo, W.; Eijkelenboom, A.; Chen, S.; Muhlestein, W.; Melton, D.A. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nat. Biotechnol.*, **2008**, 26(11), 1269-1275.
- [171] Kim, J.B.; Greber, B.; Arauzo-Bravo, M.J.; Meyer, J.; Park, K.I.; Zaehres, H.; Scholer, H.R. Direct reprogramming of human neural stem cells by OCT4. *Nature*, **2009**, 461(7264), 649-643.
- [172] Kim, J.B.; Sebastiano, V.; Wu, G.; Arauzo-Bravo, M.J.; Sasse, P.; Gentile, L.; Ko, K.; Ruau, D.; Ehrlich, M.; van den Boom, D.; Meyer, J.; Hubner, K.; Bernemann, C.; Ortmeier, C.; Zenke, M.; Fleischmann, B.K.; Zaehres, H.; Scholer, H.R. Oct4-induced pluripotency in adult neural stem cells. *Cell*, **2009**, 136(3), 411-419.
- [173] Kim, J.B.; Zaehres, H.; Wu, G.; Gentile, L.; Ko, K.; Sebastiano, V.; Arauzo-Bravo, M.J.; Ruau, D.; Han, D.W.; Zenke, M.; Scholer, H.R. Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. *Nature*, **2008**, 454(7204), 646-650.
- [174] Stadtfeld, M.; Nagaya, M.; Utikal, J.; Weir, G.; Hochedlinger, K. Induced pluripotent stem cells generated without viral integration. *Science*, **2008**, 322(5903), 945-949.
- [175] Lyssiotis, C.A.; Foreman, R.K.; Staerk, J.; Garcia, M.; Mathur, D.; Markoulaki, S.; Hanna, J.; Lairson, L.L.; Charette, B.D.; Bouchez, L.C.; Bollong, M.; Kunick, C.; Brinker, A.; Cho, C.Y.; Schultz, P.G.; Jaenisch, R. Reprogramming of murine fibroblasts to induced pluripotent stem cells with chemical complementation of Klf4. *Proc. Natl. Acad. Sci. USA*, **2009**, 106(22), 8912-8917.
- [176] Gonzalez, F.; Barragan Monasterio, M.; Tiscornia, G.; Montserrat Pulido, N.; Vassena, R.; Battle Morera, L.; Rodriguez Piza, I.; Izpisua Belmonte, J.C. Generation of mouse-induced pluripotent stem cells by transient expression of a single nonviral polycistronic vector. *Proc. Natl. Acad. Sci. USA*, **2009**, 106(22), 8918-8922.
- [177] Yu, J.; Hu, K.; Smuga-Otto, K.; Tian, S.; Stewart, R.; Slukvin, I.; Thomson, J.A. Human induced pluripotent stem cells free of vector and transgene sequences. *Science*, **2009**, 324(5928), 797-801.
- [178] Okita, K.; Hong, H.; Takahashi, K.; Yamanaka, S. Generation of mouse-induced pluripotent stem cells with plasmid vectors. *Nat. Protoc.*, **2010**, 5(3), 418-428.
- [179] Dimos, J.T.; Rodolfa, K.T.; Niakan, K.K.; Weisenthal, L.M.; Mitsumoto, H.; Chung, W.; Croft, G.F.; Saphier, G.; Leibel, R.; Golland, R.; Wichterle, H.; Henderson, C.E.; Eggan, K. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science*, **2008**, 321(5893), 1218-1221.
- [180] Maehr, R.; Chen, S.; Snitow, M.; Ludwig, T.; Yagasaki, L.; Golland, R.; Leibel, R.L.; Melton, D.A. Generation of pluripotent stem cells from patients with type 1 diabetes. *Proc. Natl. Acad. Sci. USA*, **2009**, 106(37), 15768-15773.
- [181] Miyoshi, N.; Ishii, H.; Nagai, K.; Hoshino, H.; Mimori, K.; Tanaka, F.; Nagano, H.; Sekimoto, M.; Doki, Y.; Mori, M. Defined factors induce reprogramming of gastrointestinal cancer cells. *Proc. Natl. Acad. Sci. USA*, **2010**, 107(1), 40-45.
- [182] Zhou, Q.; Brown, J.; Kanarek, A.; Rajagopal, J.; Melton, D.A. *In vivo* reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature*, **2008**, 455(7213), 627-632.
- [183] Warren, L.; Manos, P.D.; Ahfeldt, T.; Loh, Y.H.; Li, H.; Lau, F.; Ebina, W.; Mandal, P.K.; Smith, Z.D.; Meissner, A.; Daley, G.Q.; Brack, A.S.; Collins, J.J.; Cowan, C.; Schlaeger, T.M.; Rossi, D.C. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell*, **2010**, Sep 29. [Epub ahead of print]
- [184] Kim, K.; Doi, A.; Wen, B.; Ng, K.; Zhao, R.; Cahan, P.; Kim, J.; Aryee, M.J.; Ji, H.; Ehrlich, L.I.; Yabuuchi, A.; Takeuchi, A.; Cunniff, K.C.; Hongguang, H.; McKinney-Freeman, S.; Naveiras, O.; Yoon, T.J.; Irizarry, R.A.; Jung, N.; Seita, J.; Hanna, J.; Murakami, P.; Jaenisch, R.; Weissleder, R.; Orkin, S.H.; Weissman, I.L.; Feinberg, A.P.; Daley, G.Q. Epigenetic memory in induced pluripotent stem cells. *Nature*, **2010**, 467(7313), 285-290.
- [185] Polo, J.M.; Liu, S.; Figueroa, M.E.; Kulalert, W.; Eminli, S.; Tan, K.Y.; Apostolou, E.; Stadtfeld, M.; Li, Y.; Shioda, T.; Natesan, S.; Wagers, A.J.; Melnick, A.; Evans, T.; Hochedlinger, K. Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nat. Biotechnol.*, **2010**, 28(8), 848-855.
- [186] Ji, H.; Ehrlich, L.I.; Seita, J.; Murakami, P.; Doi, A.; Lindau, P.; Lee, H.; Aryee, M.J.; Irizarry, R.A.; Kim, K.; Rossi, D.J.; Inlay, M.A.; Serwold, T.; Karsunky, H.; Ho, L.; Daley, G.Q.; Weissman, I.L.; Feinberg, A.P. Comprehensive methylome map of lineage commitment from haematopoietic progenitors. *Nature*, **2010**, 467(7313), 338-342.